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13. ABSTRACT (Maximum 200 words) The objective of this research was to characterize the influence of bacterial cell-surface characteristics (exopolymers, surface charges) on adhesion to substrata. Mutants of <i>Pseudomonas aeruginosa</i> with known surface characteristics were used in adhesion tests under laminar flow in aqueous environments. Spectroscopy, microscopy, and fluorescence techniques were used to quantify the relative adhesion propensity of the various mutants. It was discovered that exopolymer is not required in large amounts for adhesion however lipopolysaccharide chemistry seems to play a role in adherence and biofilm accumulation.				
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FINAL REPORT

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GRANT TITLE: Interfacial Interactions in Microbial Bioadhesion

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OBJECTIVE: To determine the effect of changes in exopolysaccharide structure of bacteria on attachment and biofilm formation.

APPROACH: Utilize mutants of *Pseudomonas aeruginosa* with structural changes in the exopolysaccharides (alginate and lipopolysaccharide) to examine attachment in laminar flow cells by tryptophan fluorescence, direct cell counts and infrared spectroscopy, and to monitor effects of attachment on alginate synthesis by bioluminescence with a *lux-algD* reporter strain. Determine changes in electrostatic and hydrophobic properties of adherence mutants.

ACCOMPLISHMENTS: Attachment of mucoid (wild type; produces exopolymer) and of nonmucoid (mutant; reduced exopolymer production) *P. aeruginosa* cells to germanium prisms was monitored by ATR-FTIR. Both cell types attached and formed biofilms indicating that copious production of exopolymer is not required for attachment or for biofilm formation by these organisms.

Attachment and biofilm formation of *P. aeruginosa* strains O6 (wild type, complete lipopolysaccharide [LPS]), R5 (mutant, lacks almost all LPS) and A28 (mutant, LPS composition intermediate between that of O6 and that of R5) was investigated in laminar flowcells and evaluated by tryptophan fluorescence or by confocal microscopy. Strain R5 attached rapidly and formed densely packed, confluent biofilms of uniform thickness. Strain O6 attached slower and did not grow as rapidly as did strain R5; total biomass in O6 biofilms was lower than for strain R5. Microscopic appearance of the initial biofilms was (in contrast to that of strain R5) patchy with distinct hemispherical microcolonies. Strain A28 did not differ significantly in attachment or biofilm biomass accumulation than strain R5, although the microscopic appearance of the A28 biofilm was intermediate between that of R5 and that of O6.

Hydrophobic Interaction Chromatography and electrostatic Interaction Chromatography were used to investigate the cell-surface characteristics of the *P. aeruginosa* LPS mutants. Strains A28 and R5 were found to be significantly more hydrophobic and higher in surface charge than strain O6. A28 and R5 are known to lack the long-chain LPS subunit ("B-band" LPS).

CONCLUSIONS: Molecular interactions between cells and substrata involved in bacterial adherence and biofilm formation can be investigated nondestructively and in real time. Information on changes in the chemical composition of the biofilms can be acquired by FTIR spectroscopy. This approach can be used to determine environmental effects on exopolymer production and biochemistry. We have shown that exopolymer production appears not to be significant in biofilm formation by these *P. aeruginosa* strains although it certainly plays a role in the physiology of the bacterial community.

In contrast to the exopolymer data, LPS composition does appear to play an important role in attachment, in biofilm formation and in biofilm architecture in the *P. aeruginosa* strains examined. The presence of B-band LPS seems to reduce overall cell-surface charge and hydrophobicity, thereby reducing the propensity for attachment and biofilm growth on the substrata we have investigated (glass, stainless steel).

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